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Wound infection of plum fruit by airborne conidia of *Monilinia laxa*

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Abstract. Infection of non-wounded and freshly wounded detached plum fruit by airborne *Monilinia laxa* conidia on dry, humid and wet plum fruit surfaces, and by conidia and germlings that had been established on fruits under these wetness regimes, was investigated. Plum fruit (cv. Laetitia) were dusted with dry conidia in a settling tower at pit hardening, 2 weeks before harvest, at harvest and after 28 days cold storage. Non-wounded immature and mature fruit remained mostly asymptomatic, whereas non-wounded cold-stored fruit decayed readily. Wounding drastically increased infection by airborne conidia. Immature fruits were less susceptible to wound infection by airborne conidia than mature fruits. Conidia that were dispersed freshly were more successful in infecting fresh wounds than conidia that were deposited, or germlings that established, on fruits 4 days prior to wounding. This decrease in infectivity was especially pronounced on humid-incubated fruit and on wet-incubated fruit. This study clearly showed that in order to reduce the incidence of brown rot, inoculum levels on fruit approaching maturity should be reduced by sanitary practices and fungicides. Furthermore, it is essential to protect fruit, especially near-mature fruit, from being wounded.

Additional keywords: brown rot, moist incubation.

Introduction

In the stone fruit producing regions of the Western Cape province of South Africa, brown rot of nectarine and plum is considered the most destructive phase of the *Monilinia laxa* disease syndrome (Fourie and Holz 1985*a*; Schlagbauer and Holz 1987; Fourie *et al*. 2002). Several reports indicated that on both fruit types, infection is established when fruits are approaching maturity (Kable 1971; Schlagbauer and Holz 1989*a*, 1989*b*; Fourie *et al*. 2002; Fourie and Holz 2003*a*, 2003*b*). Fruit generally remained free of latent infection at the shuck fall and pit hardening stages and only developed symptoms on mature fruit. Infection studies with solitary conidia provided evidence for the importance of short-term latency of *M. laxa* on nectarine (Fourie and Holz 2003*a*), but not on plum (Fourie and Holz 2003*b*). The studies furthermore showed that the barrier capacity of skins of the two fruit types differed substantially later in the season. On nectarine, fruits skins were more readily penetrated and disease expression became more pronounced when fruit approached maturity. Maturing plum fruit, on the other hand, did not display this drastic change in the barrier capacity of fruit skins. Collectively, the findings indicate that *M. laxa* fruit rot epidemics on plum and nectarine may be driven by inoculum levels on fruit approaching maturity and by weather conditions prevailing during the preharvest and harvest period. The role of latent contamination (Jerome 1958) may thus be underestimated in the epidemiology of *M. laxa* on plum and nectarine fruit.

The mode of penetration of fruits by the brown rot fungi, *M. fructicola*, *M. laxa* and *M. fructigena*, and subsequent disease expression, have not been well documented. Infection by these pathogens is mainly associated with wounds on fruit (Byrde and Willetts 1977; Fourie and Holz 1985*b*; Xu and Robinson 2000). However, in the laboratory, penetration of fruits inoculated with conidial suspensions has been observed through undamaged surfaces, including direct penetration of the cuticle by appressoria of *M. fructicola* (Cruickshank and Wade 1992*b*) and structures such as hair sockets, lenticels and stomata (Curtis 1928; Smith 1936; Hall 1971; Byrde and Willetts 1977; Willetts and Bullock 1993). By working with airborne conidia of *M. laxa*, it was demonstrated that germlings do not penetrate nectarine (Fourie and Holz 2003*a*) or plum fruit directly (Fourie and Holz 2003*b*), but entered through stomata, lenticels and microfissures in the fruit skin. It was furthermore suggested that as the skin of plum acts as an effective barrier to *M. laxa*, more emphasis should be placed on careful handling of fruit and prevention of injuries during harvest and handling practices. However, information describing wounding and fruit rot epidemics of stone fruit by *M. laxa* is lacking. The aim of this study was

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(i) to determine the infection of fresh wounds by airborne *M. laxa* conidia on dry, humid and wet plum surfaces, and (ii) to investigate whether conidia and germlings that have been established on fruits under this range of wetness regimes can infect fresh wounds. The inoculation, incubation and wounding techniques used simulate infection under natural conditions.

Methods

Fruit

A plum orchard (cv. Laetitia) with a history of low levels of brown rot incidence was selected in the Blaauwklippen valley, Stellenbosch. Four weeks prior to the pit hardening stage, a section of this orchard was demarcated where no fungicides were applied until harvest. Sound, unblemished fruit were selected at pit hardening, 2 weeks before harvest, and at the harvest stage from these trees. Fruit obtained at harvest stage were either used, or kept under conditions simulating overseas shipment and marketing before being used (10 days at −0.5°C, 18 days at 7.5°C followed by 1 week at 23°C at ±56% R.H.). Relative humidity during this and other incubation regimes in this study was determined with a portable hygrometer (HI 9065, Hanna Instruments, Ronchi di Villafranca, Italy). Before usage, fruits were surface sterilised (30 s in 70% ethanol, 2 min in 2% sodium hypochlorite, 30 s in 70% ethanol), packed on sterile, epoxy-coated steel mesh screens (53 × 28 × 2 cm) and allowed to air-dry. Picking wounds at or near the peduncle-end were covered with petroleum jelly. In order to recognise the inoculated cheek of the fruit at a later stage, a 0.5 cm mark was made near the peduncle-end with a soft-tipped permanent marker pen. Preliminary studies showed no phytotoxic effect. Before inoculation, surface sterilised fruit were kept for at least 24 h in ethanol-disinfected Perspex (Cape Plastics) chambers (60 × 30 × 60 cm) at 22°C at ±56% R.H. to allow re-establishment of surface nutrients (Fourie and Holz 1998).

Inoculation

A virulent *M. laxa* isolate, obtained from a naturally infected nectarine fruit and shown to be sensitive to triforine, iprodione and benomyl (data not shown), was maintained in the laboratory at 22°C on a synthetic agar medium amended with sugars, minerals and malic acid at concentrations occurring in grape berry exudates (1.85 g glucose, 1.95 g fructose, 0.25 g sucrose, 0.15 g malic acid, 5 g peptone, 5 g sodium chloride, 2 g yeast extract and 15 g agar per L deionised water), or was kept on malt extract agar (MEA) slopes at 5°C in the dark. Inoculum was prepared by inoculating ripe surface-sterilised nectarines with mycelium discs or conidia obtained from fresh cultures growing on potato dextrose agar (PDA). Inoculated fruit were incubated for 10 to 14 days at 22°C on screens in humid Perspex chambers (see below) to allow infection, complete colonisation and profuse sporulation by *M. laxa*. These fruit were then kept in dry chambers at ±56% R.H. until used for inoculation, when a fruit was placed on a shelf 10 cm below the ceiling of a spore settling tower (1.5 × 1.0 × 1.5 m [length × width × height]). Conidia were blown for 1 s from the mummy with a pressure pump (Rietchle VTE 3, 3.5–4.2 m^3/h) and the lid in the ceiling closed. The conidia were allowed 10 min to settle onto the fruit, which were positioned on three screens on the floor of the tower. Previous studies have shown that by using this technique, ∼9 conidia/mm^2 were deposited on the upward facing cheeks (∼400 mm^2) of plum fruits (Fourie and Holz 2003*b*). Petri dishes with PDA were placed on the floor of the settling tower at each inoculation and percentage germination of conidia was determined after 6 h incubation at 22°C (100 conidia per Petri dish, three replicates).

Infection of fresh wounds by fresh conidia

Wounds (10 wounds per fruit, 30 fruits per sampling) were made on the marked side of fruit with a cork stopper (dome shaped to fit onto the fruit cheek), through which five needles protruded in a criss-cross pattern. The needles were 5 mm apart and inflicted wounds 1.5 mm deep. After each wounding, the wound instrument was sterilised by pressing it for five seconds onto an ethanol drenched cotton wool swab. After wounding, the fruit were kept for 1 h at low humidity (±65% R.H.). Preliminary microscopic examinations showed that within this 1 h period exudates leaked from the wound onto a small fringe of the surrounding skin and then retracted. Control fruit (30 fruit per sampling) were left unwounded. The wounded and unwounded fruit were then inoculated and incubated at 22°C under dry, humid or wet conditions. For dry incubation, Perspex chambers were kept dry (±65% R.H.). For humid incubation, Perspex chambers were lined with a sheet of chromatography paper with the base resting in deionised water to establish high relative humidity (≥93% R.H.). For wet incubation, Perspex chambers were lined with a sheet of chromatography paper as described before, and fruit were overlaid with sterile paper towels soaked with sterile deionised water. These conditions provided three different moisture regimes for the pathogen; i.e. dry conidia on dry fruit at low humidity (dry), dry conidia on dry fruit at high humidity (humid), and dry conidia on fruit covered in a film of water (wet). After 24 h, the fruit were removed from the chambers and the wet paper towels carefully removed from the wet-incubated fruit. The fruit were packed into cartons and kept for a further 10 days at 23°C under dry conditions (±56% R.H.).

Infection of fresh wounds by established inocula

Sound, unblemished fruit (30 per sampling) were inoculated and incubated in the Perspex chambers under the set of wetness regimes described above. After 24 h the fruit were removed from the chambers, the wet paper towels carefully removed from the wet-incubated fruit and the fruit air-dried. The air-dried fruit were packed into cartons and incubated for an additional 72 h at 23°C under dry conditions (±56% R.H.) to establish germling growth and penetration. The fruit were then wounded as described above. Control fruit (30 per sampling) were left unwounded. The wounded and non-wounded fruit were kept for a further 10 days before decay was assessed.

Decay assessment and statistical analyses

Inoculated fruit were inspected daily, the number of infected wounds recorded and the percentage decayed wounds per fruit (decay severity) was calculated. The incidence of decay (percentage of decayed fruit) was recorded for the non-wounded fruit. The trials were repeated twice. Analysis of variance of a completely randomised split-plot design was done using SAS Version 8.2 (SAS Institute Inc., Cary, NC). Least significant difference values were obtained and the means compared using Student's *t*-test (Snedecor and Cochran 1980).

Results

Analyses of variance on the mean decay incidence on unwounded and wounded fruit (Table 1) and on the percentage wounds that developed decay (Table 2) showed significant three-factor interactions between stage, wound and incubation treatment for decay incidence and percentage decaying wounds.

Conidia used at each inoculation were highly viable, 98–100% germinating on PDA at 6 h post inoculation (hpi). Decay incidences and percentage wounds infected are given in Tables 3 and 4, respectively. On the unwounded fruit

Table 1. Analysis of variance for effects of growth stage (G), wounding (I) and wetness regime [wet(W)/humid(H)/dry(D)] on decay (%) caused by airborne conidia of *Monilinia laxa* on surfaces of plum fruit (cv. Laetitia)

Source of variation	d.f.[A]	Sum of squares	Mean square	Significance
Model	35	196 166	5605	0.0001
G	3	53 203	17 734	0.0001
I	2	98 941	49 470	0.0001
W/H/D	2	2941	1470	0.0002
G × I	6	23 511	3919	0.0001
G × W/H/D	6	1533	256	0.1275
I × W/H/D	4	9426	2356	0.0001
G × I × W/H/D	12	6611	551	0.0002
Error	72	10 667	148	
Corrected total	107	206 832		

[A]Degrees of freedom.

Table 2. Analysis of variance for effects of growth stage (G), wounding (I) and wetness regime [wet(W)/humid(H)/dry(D)] on wound infection (%) caused by airborne conidia of *Monilinia laxa* on surfaces of plum fruit (cv. Laetitia)

Source of variation	d.f.[A]	Sum of squares	Mean square	Significance
Model	23	23 906	1039	0.0001
G	3	9603	3201	0.0001
I	1	7565	7565	0.0001
W/H/D	2	172	86	0.1573
G × I	3	2567	856	0.0001
G × W/H/D	6	542	90	0.0809
I × W/H/D	2	2248	1124	0.0001
G × I × W/H/D	6	1211	202	0.0010
Error	48	2143	45	
Corrected total	71	26 049		

[A]Degrees of freedom.

(Table 3), no decay developed on fruit at pit hardening and 2 week before harvest stages and very low levels of decay at harvest stage (0 to 6.7%). Cold-stored fruit, on the other hand, were more susceptible with fruit decaying in both the dry and humid treatments at levels of 6.7 and 3.3%, respectively. Wet-incubation increased decay incidence to 23.3%.

The ability of fresh conidia to infect fresh wounds was influenced by fruit phenology and wetness (Tables 3, 4). On immature fruit, decay at the wound sites was obvious at ~48 hpi and on mature fruit at 24 hpi. Lesions on immature fruit were restricted and leathery, but soft and expanded fast on mature fruit. At pit hardening, 26.7% of dry-incubated fruits decayed. Decay levels were, however, relatively low on fruit that were humid (6.7%) or wet-incubated (13.3%) and decay developed from a minority of the wound sites. Wounding drastically affected decay levels when fruits were wounded 2 weeks before harvest or at harvest stage. Incidences were unaffected by wetness regime and nearly all the fruit incubated under the dry, humid and

Table 3. Mean percentage of plum fruit (cv. Laetitia) at different growth stages that developed *Monilinia laxa* decay after being subjected to a differential set of inoculation, wounding and incubation treatments

Means (%) followed by different small letters indicate significant difference between treatments (within columns), whereas capital letters indicate differences between stages (within rows). Least significant difference ($P = 0.05$) = 19.78

Treatment[A,B]	Pit hardening	2 weeks before harvest	Harvest	Cold stored fruit
Unwounded				
Dry	0.0aA	0.0aA	3.3aA	6.7aA
Humid	0.0aA	0.0aA	0.0aA	3.3aA
Wet	0.0aA	0.0aA	6.7aA	23.3abAB
Fresh wounds and fresh conidia				
Dry	26.7abA	90.0cB	100.0cB	96.7dB
Humid	6.7aA	93.3cB	100.0cB	96.7dB
Wet	13.3aA	100.0cB	100.0cB	100.0dB
Fresh wounds and established inocula				
Dry	0.0aA	96.7cB	83.3cB	93.3dB
Humid	3.3aA	60.0bB	93.3cC	66.7cB
Wet	0.0aA	10.0aA	56.7bC	33.3bB

[A]Unwounded = unblemished fruit were dusted with conidia in a spore settling tower and incubated; fresh wounds and fresh conidia = fruits were wounded, dusted with conidia and incubated; fresh wounds and established inocula = fruits were dusted with conidia, incubated and freshly wounded.
[B]Dry = fruit incubated dry (±65% R.H.); humid = fruit incubated at high humidity (≥93% R.H.); wet = fruit overlaid with wet paper towels.

Table 4. Mean percentage of wounds infected by *Monilinia laxa* on plum fruit (cv. Laetitia) at different growth stages after being subjected to a differential set of inoculation, wounding and incubations treatments

Means (%) followed by different small letters indicate significant difference between treatments (within columns), whereas capital letters indicate differences between stages (within rows). Least significant difference ($P = 0.05$) = 10.97

Treatment[A,B]	Pit hardening	2 weeks before harvest	Harvest	Cold stored fruit
Fresh wounds and fresh conidia				
Dry	4.3aA	27.3bB	40.3cBC	31.3bB
Humid	0.7aA	31.0bB	41.3cBC	28.3bB
Wet	1.7aA	34.0bcB	68.3dD	52.0cC
Fresh wounds and established inocula				
Dry	0.0aA	22.3bB	17.0aB	23.7bB
Humid	0.3aA	8.3aA	19.7abB	11.3aAB
Wet	0.0aA	1.0aA	7.6aA	3.3aA

[A]Fresh wounds and fresh conidia = fruits were wounded, dusted with conidia in a spore settling tower and incubated; fresh wounds and established inocula = fruits were dusted with conidia, incubated and freshly wounded.
[B]Dry = fruit incubated dry (±65% R.H.); humid = fruit incubated at high humidity (≥ 93% R.H.); wet = fruit overlaid with wet paper towels.

wet conditions developed decay. Wetness had no significant effect on the percentage wound sites that developed decay on fruit inoculated 2 weeks before harvest, but the percentage was significantly increased on fruit inoculated at harvest by incubation under wet conditions. A similar trend was found on cold stored fruit.

The ability of established inocula to infect fresh wounds differed from the trend described for fresh inoculum. Firstly, on immature fruit, decay at the wound sites became visible ~72 hpi and on mature fruit, 60 hpi. Secondly, at pit hardening, none of dry- or wet-incubated fruits decayed and very low decay and wound infection levels were observed on fruit that were humid-incubated (Tables 3, 4). On cold stored fruit and fruit from the 2 week before harvest and harvest stages, significantly more wound sites on fruits kept dry, than on humid or wet fruit, developed decay (Table 4). This was also reflected in the percentage fruit decay (Table 3).

Discussion

The inoculation technique used in this study simulates natural dispersal of airborne conidia. Fruits were either not wounded, or had 10 small, artificially inflicted wounds, which provided ample opportunity for direct penetration or contact between wounds and solitary conidia or germlings was provided on dry, humid or wet plum fruit. Such conditions normally prevail in the orchard, fruit bins or storage cartons (Fourie and Holz 1992). Under these conditions, non-wounded, immature and mature fruit mostly remained asymptomatic, whereas non-wounded cold stored fruit decayed, especially when kept wet. Wounding drastically increased infection by airborne conidia of *M. laxa*, which confirmed previous observations (Fourie and Holz 1985*b*, 1987) on the necessity of wounds for infection after inoculation with spore suspensions in the laboratory. Airborne conidia (Fourie and Holz 2003*b*) and conidia suspended in droplets (Curtis 1928; Smith 1936; Hall 1971; Byrde and Willetts 1977; Willetts and Bullock 1993) penetrated plum fruits directly through natural openings, but these infections did not always lead to fruit decay.

Immature fruits were less susceptible to wound infection by airborne *M. laxa* conidia than mature fruits. There are several possible explanations for this. Firstly, the inability of solitary *M. laxa* conidia to infect immature plum fruits has been correlated with poor growth caused by substances in exudates, wax layers or other skin components (Fourie and Holz 2003*b*). Phenols, particularly chlorogenic and caffeic acids, are high in resistant immature peach genotypes and decline with fruit maturity (Bostock *et al*. 1999). Working with *Botrytis cinerea*, Fourie and Holz (1998) showed that prior to the period of rapid cell enlargement, growth of this fungus on raised slides was inhibited by plum exudates. Secondly, germlings of *M. laxa* need an external supply of nutrients for germ tube elongation and penetration of host surfaces since conidia of the brown rot fungi contain insufficient reserves (Willetts and Bullock 1993). Sugar concentrations in the exudates of plum fruit are low prior to pit hardening (Fourie and Holz 1998). At corresponding concentrations, glucose, fructose and sucrose did not influence growth of *B. cinerea* in a mineral medium. Fungal growth was only enhanced when either of the reducing sugars or sucrose was supplied in excess of 0.27 and 0.14 mM, respectively. During the 2 weeks prior to harvest, total sugar in plum exudates was near these values. These effects were shown by solitary *M. laxa* conidia grown on humid and wet fruit (Fourie and Holz 2003*b*).

Freshly dispersed conidia were more successful in infecting fresh wounds than conidia that were deposited, or germlings that had established on fruits 4 days prior to wounding. This decrease in infectivity was especially pronounced on humid- and even more on wet-incubated fruit. Fluorescence microscopy studies of the behaviour of *M. laxa* on unwounded nectarine and plum (Fourie and Holz 2003*a*, 2003*b*) surfaces showed that solitary conidia formed germ tubes within 3 hpi on both humid and wet fruit. Germination rates were higher and germ tube growth was more extensive on wet fruit. Wetness, however, had a negative effect on survival of the pathogen. Different criteria showed that free water on the fruit surface drastically reduced the viability of conidia and germlings. The deleterious effect of increased wetness on the survival of conidia, therefore, resulted in lower decay levels on fruit that were wounded 72 h after wet- or humid-incubation. Decay levels on dry incubated fruit that were wounded after incubation were comparable with that observed on fruit wounded prior to inoculation, except on plum fruit from the pit hardening stage. These results, therefore, agree with conclusions by Naqvi and Good (1957) that very humid conditions were more detrimental to conidium survival than very dry conditions.

Germination and germ tube growth on fruit incubated under relatively dry conditions (±65% R.H.) were not microscopically studied. Previous studies with nectarine and plum fruits (Fourie and Holz 2003*a*, 2003*b*) have shown that airborne *M. laxa* conidia seldom land on stomata, lenticels or micro-fissures. Germlings usually entered these structures when they grew in proximity to them. The tendency to grow towards a specific site and to penetrate was furthermore enhanced by fruit wetness. Given the fact that wounded fruits that were kept dry decayed, the event of germination and germ tube growth at the wound site on these fruits must be accepted. Preliminary microscopic examinations showed that exudates exuded from the wound onto a small fringe of the surrounding skin and then withdrew within an hour. Wound infection on dry fruit indicates that the microclimate around the wound site may be conducive to germination. In the event of conidia deposited prior to wounding, exudate withdrawal may also carry the non-germinated conidia into the wound site, thereby enhancing infection. Wound exudates would, therefore, provide the free water and carbon sources, such as

glucose, which are most important for successful infection (Wade and Cruickshank 1992; Xu and Robinson 2000).

It has been suggested (Fourie *et al*. 2002; Fourie and Holz 2003*a*, 2003*b*) that *M. laxa* fruit rot epidemics on plum and nectarine are driven by inoculum levels on fruit approaching maturity and by weather conditions prevailing during the preharvest and harvest period. Latent contamination (Jerome 1958) may, therefore, be of major importance in the epidemiology of *M. laxa* on plum fruit. This study clearly showed that in order to reduce the incidence of brown rot, inoculum levels on fruit approaching maturity should be reduced by fungicide applications and sanitation practices, such as the immediate removal of discarded or decayed fruit from orchards. Furthermore, careful harvesting and handling are essential to protect fruits from being wounded, and condensation on fruit in storage should be prevented as wetness enhanced infection of wounded and unwounded fruit.

References

Bostock RM, Wilcox SM, Wang G, Adaskaveg JE (1999) Suppression of *Monilinia fructicola* cutinase production by peach fruit surface phenolic acids. *Physiological and Molecular Plant Pathology* **54**, 37–50. doi: 10.1006/pmpp.1998.0189

Byrde RJW, Willetts HJ (1977) 'The brown rot fungi of fruit: their biology and control.' 1st edn. (Pergamon Press Ltd: Oxford)

Cruickshank RH, Wade GC (1992*b*) Production of appressoria by *Monilinia fructicola. Mycological Research* **96**, 425–428.

Curtis KM (1928) The morphological aspect of resistance to brown rot in stone fruit. *Annals of Botany* **42**, 39–68.

Fourie JF, Holz G (1985*a*) Postharvest fungal decay of stone fruit in the South-Western Cape. *Phytophylactica* **17**, 175–177.

Fourie JF, Holz G (1985*b*) Artificial inoculation of stone fruit with *Botrytis cinerea*, *Monilinia laxa* and *Rhizopus stolonifer*. *Phytophylactica* **17**, 179–181.

Fourie JF, Holz G (1987) Infection and decay of stone fruit by *Botrytis cinerea* and *Monilinia laxa* at different stages after anthesis. *Phytophylactica* **19**, 45–46.

Fourie JF, Holz G (1992) Effect of free moisture on the development of post-harvest decay. In 'Proceedings of the Conference on Refrigeration in the Production, Storage and Distribution of Perishable Products'. pp. F2.1–F2.10.

Fourie JF, Holz G (1998) Effects of fruit and pollen exudates on growth of *Botrytis cinerea* and infection of plum and nectarine fruit. *Plant Disease* **82**, 165–170.

Fourie PH, Holz G (2003*a*) Germination of dry, airborne conidia of *Monilinia laxa* and disease expression on nectarine fruit. *Australasian Plant Pathology* **32**, 9–18. doi: 10.1071/AP02063

Fourie PH, Holz G (2003*b*) Germination of dry, airborne conidia of *Monilinia laxa* and disease expression on plum fruit. *Australasian Plant Pathology* **32**, 19–25. doi: 10.1071/AP02066

Fourie PH, Holz G, Calitz FJ (2002) Occurrence of *Botrytis cinerea* and *Monilinia laxa* on nectarine and plum in Western Cape orchards, South Africa. *Australasian Plant Pathology* **31**, 197–204. doi: 10.1071/AP02007

Hall R (1971) Pathogenicity of *Monilinia fructicola* Part II. Penetration of peach leaf and fruit. *Phytopathologische Zeitschrift* **72**, 281–290.

Jerome SMR (1958) Brown rot of stone fruits. Latent contamination in relation to spread of the disease. *The Journal of the Australian Institute of Agricultural Science* **24**, 132–140.

Kable PF (1971) Significance of short-term latent infections in the control of brown rot in peach fruits. *Phytopathologische Zeitschrift* **70**, 173–176.

Naqvi SHZ, Good HM (1957) Studies of the aging of conidia of *Monilinia fructicola* (Wint.) Honey. I. Germination rates and longevity. *Canadian Journal of Botany* **35**, 635–645.

Schlagbauer HE, Holz G (1987) Blossom blight and brown rot of stone fruit caused by *Monilinia laxa* in the Cape Province of South Africa. *Phytophylactica* **19**, 513–514.

Schlagbauer HE, Holz G (1989*a*) Occurrence of latent *Monilinia laxa* infections on plums, peaches and apricots. *Phytophylactica* **21**, 35–38.

Schlagbauer HE, Holz G (1989*b*) Penetration of plums by *Monilinia laxa* and histology of a defense reaction. *Phytophylactica* **21**, 39–43.

Smith MA (1936) Infection studies with *Sclerotinia fructicola* on brushed and non-brushed peaches. *Phytopathology* **26**, 1056–1060.

Snedecor GW, Cochran WG (1980) 'Statistical methods.' 7th edn. (Iowa State University Press: Ames)

Wade GC, Cruickshank RH (1992) Rapid development of resistance of wounds on immature apricot fruit to infection with *Monilinia fructicola. Journal of Phytopathology* **136**, 89–94.

Willetts HJ, Bullock S (1993) Cytology, histology, and histochemistry of fruit infection by *Monilinia* species. In 'Handbook of cytology, histology, and histochemistry of fruit tree diseases'. (Ed. AR Biggs) pp. 113–136. (CRC Press, Inc.: Boca Raton)

Xu X-M, Robinson JD (2000) Epidemiology of brown rot (*Monilinia fructigena*) on apple: infection of fruits by conidia. *Plant Pathology* **49**, 201–206. doi: 10.1046/j.1365-3059.2000.00437.x

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